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Applied Microbiology and Planetary Quarantine Section
Phoenix Laboratories
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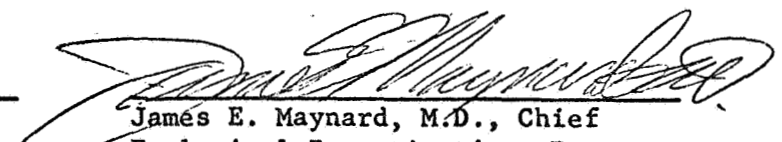
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1. As part of the study to evaluate certain aspects of the swab-rinse technique, several different swab materials were compared for effectiveness in removing and recovering naturally occurring microbial contamination from surfaces. Utilizing the methodology described in Q.R. 31, a single experienced individual swabbed 15 sets of four 2 x 2" stainless steel strips with moistened dacron swabs and 15 sets with moistened rayon swabs. The removal and recovery values from these experiments were compared with each other and with the results obtained previously with moistened cotton swabs and dry cotton swabs. The comparative values are presented in Figure 1. Statistical tests of the differences between observed values demonstrated that both dry cotton and moistened dacron swabs effected significantly higher removal ($P < .05$) than either wet cotton or moistened rayon swabs. Tests of differences between recovery values showed recovery with moistened dacron to be significantly higher than that with dry cotton. Based on these results, as well as the subjective observation that dacron swabs retain their integrity during swabbing better than cotton swabs, it is suggested that dacron swabs may be the swab of choice for microbiological assays of space hardware.

As a result of finding significant differences between the removal values achieved by individuals experienced in the use of the swab-rinse technique and inexperienced individuals (Q.R. 32) a study was initiated to investigate the effect of training and experience on the subsequent performance of inexperienced individuals. An individual who achieved very poor removal (71%) in the initial test was selected for study. This individual was again instructed in the swabbing technique and was then asked to observe an experienced individual perform the swabbing procedure on a single strip. The subject was directed to swab a single strip while suggestions were made for technique improvement. The subject was then asked to observe the experienced individual swab a second strip. This procedure was repeated for six strips on each of five different days. The results achieved by the subject during these tests were compared with the results achieved by the experienced individual (Table 1) and no significant difference between either removal or recovery values was detected. The inexperienced individual performed as well as the experienced individual on the first day and no trend of subsequent improvement was noted. This training experiment will be repeated with other subjects from the inexperienced group.

2. A biodetection grinder developed and built at the Marshall Space Center was delivered to the Phoenix Laboratories for use in the study of buried microbial contamination. Experiments were conducted with the grinder and it was determined that with several modifications, the unit will provide a useful means of assaying a variety of materials. These modifications are being incorporated into a new unit which will replace the one presently in Phoenix. When the new grinder arrives, studies will be initiated to determine the optimum particle size for detecting buried microorganisms.

At a meeting with representatives from Exotech, it was decided that efforts would be made by their firm to identify those materials and components on future spacecraft in which the concentration of buried

microbial contamination should be known. Assays of these items will be of highest priority in future work on buried contamination at this laboratory.

3. Studies of the naturally occurring bacterial spore population from Cape Kennedy soil were continued. Strips were exposed to 125 C in the standard manner (Q.R. 31) for intervals of 7, 9, 16, 24, and 40 hrs, and survivors were recovered in the standard broth medium. Positive tubes were streaked for isolated colonies on the standard agar recovery medium. Isolates were chosen on the basis of differing colonial morphology and were identified to species. The results are listed in Table 2. B. firmus and B. lentus were the only two species found in the series of 20 soil isolates. Both organisms have been detected consistently on spacecraft assembled at Cape Kennedy (Table 3).

One additional isolate was obtained from the 48 hr. sample of the FN-MPN determination reported last quarter. No identification has yet been made due to the extremely slow growing nature of the organism. For example, only scant growth along with the initial line of inoculum was observed after one week's incubation of a streak plate from the positive 48 hr. FN-MPN tube. Subsequent transfers of the isolate, hereafter referred to as "Bacillus 125-48", to fresh media have not improved the growth rate appreciably. Attempts were made to sporulate the organism on TSA supplemented with yeast extract and soluble starch (Q.R. 32), TAM Sporulation Medium, AK#2 Sporulation Medium, and SSM-10 Sporulation Medium (liquid and with 1.5% agar - Q.R. 25). Incubation was conducted at 25 C to lessen dehydration of the solid media during extended incubation times. Approximately 20-30% sporulation was obtained with TAM and AK#2 after 30 days of incubation. Very few spores were noted with the SSM-10 agar medium and TSA, and Bacillus 125-48 did not grow in the SSM-10 liquid medium. The spores are spherical, 1.5 μ in diameter, and have a rough surface with projections slightly resembling "sea mines" at 1000X under a light microscope. Scanning electron photomicrographs of Bacillus 125-48 spores, obtained at the Sandia Laboratories, revealed a geometrically patterned surface of ridges predominantly in five-sided arrangements with irregular corners of the ridges forming outward protrusions from the spore surface. Heat resistance testing of this organism is currently underway and will be reported next quarter. Preliminary observations have indicated a high level of heat resistance at 125 C.

4. Last quarter a D_{125C} value of 13.9 hours was reported for the naturally occurring bacterial spore population from Cape Kennedy soil. This value was obtained from plate count data obtained at heating intervals from 0.5 to 9.0 hr. Since the estimated end-point of the population was approximately 50 hr., an additional 125 C dry heating test was conducted at sample intervals of 4, 12, 16, 24, and 32 hr. Two carrier substrates were used in this experiment. The first was the standard 1/2 in.² stainless steel strip and the second, an aluminum foil disc apparatus used by the Sandia Laboratories for dry heat testing system (M. C. Reynolds, et. al., "A study of the effectiveness of thermaradiation sterilization", Sandia Laboratories, SC-RR-70-432, June 1970). All sub-

strates were inoculated and treated in the usual manner before heating (Q.R. 31). Four units of each type of inoculated substrate were suspended at each interval and post-heating assays were done according to each respective standard method. The plating medium used was TSA supplemented with 0.2% yeast extract and 0.1% soluble starch. Plates were incubated at 32 C and counts were made at 48 hr., 5 days, and 7 days. Figure 2 shows the results. Best fit lines and D_{125C} values were calculated from mean plate count values at each interval ignoring the unheated control (N_0) value. Ranges shown are mean values of duplicate platings of each substrate unit. The D_{125C} values presented here (23.9 and 20.5 hr.) probably are more accurate reflections of the actual resistance of the spore population than the value of 13.9 hr. reported earlier. The latter value was derived from data points heavily weighted in the early portion of the survivor curve (0.5 to 9.0 hr.).

5. At the request of the Planetary Quarantine Officer, a cooperative study was initiated at the Sandia Laboratories to examine the possible synergistic effect of 125 C dry heat plus gamma radiation (thermoradiation) on the naturally occurring bacterial spore populations. In the past, such synergism has been demonstrated with B. subtilis var. niger as the test organism.

Aluminum foil discs (see above, section 4) were inoculated with 0.1 ml of the soil suspension in 95% ethanol and were stored overnight over Drierite in vacuo. Exposure method, humidity control, Sandia gamma irradiation facility (Cobalt 60), and recovery method are described by Reynolds (ibid.). Two changes in the methodology were the use of TSA supplemented with 0.1% soluble starch and 0.2% yeast extract instead of TSA alone and incubation of plates at 35 C for one week instead of 72 hr. The air supplied to the oven in all runs was controlled at 30% relative humidity. Three experiments were conducted; exposure to 125 C alone, 77 Krads/hr gamma irradiation alone, and thermoradiation. The composite results are shown in Figure 3. D-values were calculated from best fit lines of mean plate count values at each interval ignoring the N_0 value. The thermoradiation experiment shown was composed of two runs with seven sampling intervals each, i.e., each data point represents the mean value of 8 aluminum discs. The remaining two experiments were single runs representing the mean value of 4 discs at each interval. Also, it must be emphasized that the 77 Krads/hr was the rate of irradiation, not the total dosage. Five minutes were required during each interval for lowering and raising the radiation source for sample removal. Thus, the thermoradiation curve with 20 minute intervals represents a corrected dose rate of 61.8 Krads/hr while the radiation alone curve with hourly intervals represents a corrected dose rate of 71 Krads/hr.

The D_{125C} values for heat alone and thermoradiation was 30 hr. and 1.0 hr., respectively. The D-value for radiation alone was 2.75 hr. The gamma inactivation curve in Figure 3 was essentially linear, suggesting that the radiation resistance of the naturally occurring spore population was relatively homogeneous when compared to thermal inactivation or thermoradiation.

6. The Planetary Quarantine Officer requested that examination be made of naturally occurring bacterial spore population in dust collected by vacuum cleaners in various spacecraft assembly and testing areas at Cape Kennedy. Such a sample of 397 gm consisting of dust, lint, and paper and metal debris was collected in Hangar A0 and shipped to Phoenix for sieve processing (Q.R. 32). After processing through the sieve series in a completely dry state, 5.4 gm of dust passing the 43 μ stage was obtained. One-tenth gm of this material was suspended in 95% ethanol, diluted appropriately, and plated with TSA supplemented with yeast extract and soluble starch. The bacterial spore count was 7.8×10^4 /gm., which is considerably less than the titer of soil (Q.R. 32) processed in the same manner (3.5×10^7 /gm.). Results of thermal resistance testing on this spore population will be reported later.
7. Microbiological studies were performed on the Apollo 14 spacecraft during its assembly and testing at the Kennedy Space Center. The levels of microbial contamination present on the Command Module (CM-110), Instrument Unit (IU), Saturn S-4B) and the Spacecraft Lunar Module Adapter (SLA) are presented in Table 4. The interior surfaces of the SLA were found to be contaminated with higher levels of microorganisms than has been observed with prior Apollo spacecraft (Q.R. 26, 27, 28, and 30). Also, a higher number of bacterial spores was detected on the CM of Apollo 14, than was found on the CM of Apollo 12 or 13 (Q.R. 28 and 30).

Table 5 shows the quantitative data for the interior and exterior surfaces of the ascent and descent stages of the Lunar Module 8 (LM-8). The level of aerobic mesophilic microorganisms on the surfaces of the interior ascent stage (LAI), exterior ascent stage (LAE), and exterior descent stage (LDE) of the LM-8 of Apollo 14 was greater than those detected on similar areas of the Lunar Modules of Apollo 12 or 13. Bacterial spore counts also were higher than those found on the LAI, LAE of Apollo 12, and the LAI, LAE, and LDE of Apollo 13.

The surfaces of the IU, S-4B and SLA showed a higher percentage of bacterial spores than were detected on the CM surfaces (Table 6). Higher percentages of bacterial spores and molds were found on the surfaces of the LAE and LDE than on the LAI (Table 7). These data correspond with those obtained from previous Apollo spacecraft.

Table 8 shows a comparison of the levels of microbial contamination detected on the Apollo 10, 11, 12, 13, and 14 spacecraft. The levels of the aerobic mesophilic microorganisms per square foot of surface for each of the four component parts were relatively consistent from Apollo 10 to Apollo 14.

A comparison of the levels of microbial contamination of the Lunar Modules is presented in Table 9. Higher numbers of microorganisms (ca. 1 log per square foot) were found on the LAE of Apollo 14 than on Apollo 11, 12, and 13, but were similar to Apollo 10. The levels of contamination of the LAE of Apollo 14 was 1 log greater than that found on the previous four Lunar Modules. Also, the surfaces of the Apollo 14

LDE evidenced 1-2 logs higher contamination levels than the LDE components of earlier Lunar Modules. Similar increases in the levels of aerobic spores also were noted. These data (Tables 8 and 9) indicate that the Apollo 14 spacecraft, from a microbiological standpoint, was the most contaminated Apollo spacecraft flown to date.

Nine genera of fungi were isolated from Apollo 14 (Table 10). The predominant types were Bipolaris, Curvularia, Drechslera, and Penicillium. A genus of fungi, Chaetomium, which had not been detected before, also was isolated. A total of 20 genera of molds have been isolated from the five Apollo spacecraft studied.

Concern has been expressed by some investigators as to the length of incubation and kind of medium used to recover molds from spacecraft surfaces as described by the NASA Standard Procedures. In a previous report (Q.R. 24), it was shown that extending incubation from 72 hours to 21 days at 32 C did not significantly increase the number of mold colonies. Table 11 shows the data obtained when two types of media were compared. One was Trypticase Soy Agar, and the other, Mycophil Agar, a selective medium for molds. The results from three Apollo spacecraft studies indicate that the use of Mycophil Agar offers no quantitative advantage in the microbiological assay.

A total of approximately 1150 bacterial colonies were isolated from the Apollo 14 spacecraft. These isolates are being identified and results will be reported during the next quarter.

8. The relative effectiveness of the Reyniers slit sampler and the membrane filter in quantitating airborne fungi was investigated. Samples were collected simultaneously by both units located side-by-side on each of 20 days in the Spacecraft Bioassay Laboratory and on each of 14 days in the Manned Spacecraft Operations Building (MSOB). The mean concentrations detected by the two samplers were comparable in both areas as can be seen in Tables 12 and 13. To determine the degree of agreement between the two methods on a day-to-day basis, the data were subjected to regression analysis. Because the Reyniers device sampled twice as much air as the membrane filter during each sampling period, the ideal equation for the regression line would be $y = 2x + 0$; where y is the number of colonies collected with the Reyniers and x is the number of colonies collected on the membrane filter. The equation for the regression line based on MSOB data was $y = 1.50x + .17$, demonstrating consistent relative agreement as evidenced by the line passing very near the origin and a high correlation coefficient ($r = .92$). It was found, however, that the Reyniers samples regularly detected only 75% of the concentration measured by the membrane filters. The data from the laboratory area did not show the same degree of relationship. The equation for the regression line was $y = .90x + 11.18$, indicating that the line did not pass near the origin and that the Reyniers detected less than 50% of the concentration detected on the membrane filters. The correlation coefficient also was found to be lower ($r = .71$).

Because the two samplers did not perform comparably in different environments, it was concluded that the ability of membrane filters to quantitatively detect airborne fungi is different from that of the Reyniers slit sampler. Since in both areas the Reyniers detected only 50-75% of the levels of fungi found on the membrane filters, it is suggested that the membrane filter technique provides superior quantitative sampling.

In accordance with a request from the Planetary Quarantine Officer, personnel from the Spacecraft Bioassay Unit, Cape Kennedy, traveled to the Jet Propulsion Laboratory, Pasadena, California, on three separate occasions to participate in the microbiological assaying of the two Mariner-Mars 1971 spacecraft. At each sampling period, approximately half of the cotton swab samples were assayed by the USPHS microbiologist and the other half by JPL personnel. When the Proof Test Module (PTM) and the two Mariner 1971 spacecraft were moved to Cape Kennedy, the USPHS Spacecraft Bioassay Unit provided laboratory facilities, materials and work space for JPL personnel. Additional assistance was given JPL in assaying the PTM spacecraft. Microbiological assays of the two Mariner-Mars 1971 spacecraft at Cape Kennedy will be done as described by the "Mariner-Mars 1971 Microbiological Assay and Monitoring Plan". To insure independent verification of the microbiological assays, approximately half of the samples, at each sampling period, will be assayed by the USPHS personnel.

The evaluation of a terminal sterilization process for unmanned lander spacecraft has been initiated. A combination Vertical Flow Clean Bench/Dry Heat Oven was installed at Cape Kennedy, and thermal profiles were established. Preliminary experiments are being performed and the results will be reported later.

TABLE 1. COMPARISON OF SWAB-RINSE RESULTS ACHIEVED BY INSTRUCTOR AND TRAINEE # 1.

Individual	No. of Samples	Removal		Recovery	
		Mean %	Coefficient of Variation %	Mean %	Coefficient of Variation %
Instructor	30	89	4	65	18
Trainee # 1	30	86	5	57	21

TABLE 2. IDENTIFICATION OF 20 HEAT RESISTANT SPORE ISOLATES FROM CAPE
KENNEDY SOIL SUSPENSION.

Interval at 125 C	Number of Isolates	
	<u>Bacillus firmus</u>	<u>Bacillus lentus</u>
7 hr.	3	1
9 hr.	3	-
16 hr.	3	1
24 hr.	3	-
40 hr.	-	6

TABLE 3. NUMBER AND PERCENTAGE OF B. FIRMUS AND B. LENTUS DETECTED
ON APOLLO AND MARINER '69 SPACECRAFT.

Spacecraft	Total No. of <u>Bacillus</u> spp.	No. of <u>B. firmus</u>	% <u>B. firmus</u>	No. of <u>B. lentus</u>	% <u>B. lentus</u>
Apollo 10	61	5	8.2	8	13.1
Apollo 11	80	3	3.8	4	5.0
Apollo 12	107	9	8.4	8	7.5
Apollo 13	33	4	12.1	4	12.1
Mariner '69	103	17	16.5	0	0.0

TABLE 4. LEVELS OF MICROBIAL CONTAMINATION DETECTED ON THE APOLLO 14 COMMAND MODULE (CM-110), INSTRUMENT UNIT (IU), SATURN S-4B (S-4B), AND THE SPACECRAFT LUNAR MODULE ADAPTER (SLA).

Source ¹	Date Sampled	Area Sampled ² (sq. in.)	No. Microorganisms per square foot			
			Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores
CM-110	1-11-71	60	4.9×10^4	1.7×10^4	7.2×10^1	1.2×10^1
	1-16-71	60	6.9×10^4	2.4×10^4	1.4×10^2	7.2×10^1
	1-23-71	60	3.7×10^4	1.9×10^4	1.7×10^2	9.6×10^1
	1-30-71	60	1.1×10^5	4.0×10^4	2.0×10^2	1.2×10^1
IU	1-16-71	60	2.5×10^4	3.2×10^3	9.8×10^2	2.5×10^2
	1-23-71	60	2.4×10^4	1.3×10^4	9.0×10^2	1.1×10^2
	1-28-71	60	4.5×10^4	9.3×10^3	1.6×10^3	4.0×10^2
S-4B	1-16-71	60	3.8×10^4	6.3×10^3	1.3×10^3	2.3×10^2
	1-23-71	60	7.7×10^4	1.1×10^4	2.0×10^3	2.8×10^2
	1-28-71	60	2.9×10^4	8.7×10^3	1.8×10^3	6.4×10^2
SLA	1-16-71	60	2.4×10^2	4.8×10^1	1.2×10^1	0.0
	1-23-71	60	8.4×10^1	6.0×10^1	0.0	0.0
	1-27-71	60	2.3×10^2	6.0×10^1	4.8×10^1	0.0

¹ Samples were taken from the interior surfaces of the spacecraft located at Launch Complex 39 A.

² Swab-rinse technique.

TABLE 5. LEVELS OF MICROBIAL CONTAMINATION DETECTED ON LUNAR MODULE-8 (APOLLO 14).

Source	Date Sampled ²	Area Sampled ¹ (sq. in.)	No. Microorganisms per square foot		
			Aerobes	Anaerobes	Aerobic Spores Anaerobic Spores
Ascent Stage (interior)	1-16-71	60	6.2 x 10 ⁴	2.2 x 10 ⁴	1.7 x 10 ² 3.6 x 10 ¹
	1-23-71	60	4.9 x 10 ⁴	3.6 x 10 ⁴	2.6 x 10 ² 1.2 x 10 ²
	1-27-71	60	2.1 x 10 ⁵	1.1 x 10 ⁵	5.0 x 10 ² 2.4 x 10 ¹
Ascent Stage (exterior)	1-16-71	60	2.3 x 10 ⁴	4.5 x 10 ³	2.9 x 10 ² 7.2 x 10 ¹
	1-23-71	60	1.4 x 10 ⁴	1.6 x 10 ³	6.0 x 10 ¹ 2.4 x 10 ¹
	1-27-71	60	2.6 x 10 ⁴	3.7 x 10 ³	2.6 x 10 ² 2.4 x 10 ¹
Descent Stage (exterior)	1-16-71	60	2.2 x 10 ⁴	2.1 x 10 ⁴	1.8 x 10 ² 6.0 x 10 ¹
	1-23-71	60	1.8 x 10 ⁵	1.3 x 10 ⁵	3.6 x 10 ¹ 1.2 x 10 ¹
	1-27-71	60	1.2 x 10 ⁵	2.9 x 10 ⁴	4.8 x 10 ² 4.8 x 10 ¹

¹ Swab-rinse technique.

² Samples were taken while modules were located at Launch Complex 39 A.

TABLE 6. COMPARATIVE LEVELS OF AEROBIC BACTERIAL SPORES AND MOLDS DETECTED ON THE APOLLO 14 COMMAND MODULE (CM-110), INSTRUMENT UNIT (IU), SATURN S-4B, AND ON THE SPACECRAFT LUNAR MODULE ADAPTER (SLA).

Source	Date Sampled ³	Area Sampled ¹ (sq. in.)	Percent ²	
			Aerobic Bacterial Spores	Molds
Command Module CM-110	1-16-71	60	0.21	0.0
	1-23-71	60	0.45	0.0
	1-30-71	60	0.19	0.01
Instrument Unit	1-16-71	60	3.98	5.29
	1-23-71	60	3.71	2.57
	1-28-71	60	3.61	0.29
SLA	1-16-71	60	5.00	0.0
	1-23-71	60	0.0	0.0
	1-27-71	60	21.00	0.0
S-4B	1-16-71	60	3.54	2.07
	1-23-71	60	2.63	1.49
	1-28-71	60	6.39	0.33

¹ Swab-rinse technique.

² Percentage of total aerobic mesophilic microorganisms.

³ Samples were taken while spacecraft was isolated at Launch Complex 39 A.

TABLE 7. LEVELS OF AEROBIC BACTERIAL SPORES AND MOLDS DETECTED ON SURFACES
OF THE ASCENT AND DESCENT STAGES OF LUNAR MODULE-8.

Source	Date Sampled ³	Area Sampled ¹ (sq. in.)	Percent ²	
			Aerobic Bacterial Spores	Molds
Ascent Stage (interior)	1-16-71	60	0.27	0.00
	1-23-71	60	0.54	0.10
	1-27-71	60	0.24	0.005
Ascent Stage (exterior)	1-16-71	60	1.27	0.37
	1-23-71	60	0.43	0.43
	1-27-71	60	1.00	0.27
Descent Stage (exterior)	1-16-71	60	0.83	0.39
	1-23-71	60	0.02	0.00
	1-27-71	60	0.39	0.03

¹ Swab-rinse technique.

² Percentage of total aerobic mesophilic microorganisms.

³ Samples were taken while modules were located at Launch Complex 39 A.

TABLE 8. COMPARISON OF THE LEVELS OF MICROBIAL CONTAMINATION DETECTED ON COMPONENTS OF THE APOLLO 10, 11, 12, 13, AND 14 SPACECRAFT.

Source	No. Microorganisms per square foot ¹				Percent ²	
	Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores	Aerobic Spores	Molds
Command Module						
Apollo 10	2.1 x 10 ⁴	1.3 x 10 ⁴	1.7 x 10 ²	2.1 x 10 ¹	0.80	0.02
Apollo 11	2.7 x 10 ⁴	1.6 x 10 ⁴	1.3 x 10 ²	8.8 x 10 ¹	0.46	0.07
Apollo 12	2.9 x 10 ⁴	1.4 x 10 ⁴	4.0 x 10 ¹	2.4 x 10 ¹	0.14	0.0
Apollo 13	4.1 x 10 ⁴	1.8 x 10 ⁴	5.2 x 10 ¹	1.2 x 10 ¹	0.13	0.02
Apollo 14	7.1 x 10 ⁴	2.8 x 10 ⁴	1.7 x 10 ²	6.0 x 10 ¹	0.24	0.005
Instrument Unit						
Apollo 10	1.5 x 10 ⁴	2.7 x 10 ³	1.9 x 10 ³	2.7 x 10 ²	12.94	3.95
Apollo 11	7.6 x 10 ³	3.7 x 10 ³	1.3 x 10 ³	1.6 x 10 ²	17.33	7.79
Apollo 12	2.0 x 10 ⁴	4.6 x 10 ³	6.3 x 10 ²	2.6 x 10 ²	3.16	0.84
Apollo 13	1.0 x 10 ⁴	1.7 x 10 ³	5.4 x 10 ²	1.4 x 10 ²	5.46	2.05
Apollo 14	3.1 x 10 ⁴	8.5 x 10 ³	1.2 x 10 ³	2.5 x 10 ²	3.73	2.19
S-4B						
Apollo 10	2.1 x 10 ⁴	3.3 x 10 ³	3.1 x 10 ³	3.2 x 10 ²	14.66	1.97
Apollo 11	9.6 x 10 ³	3.0 x 10 ³	1.9 x 10 ³	4.4 x 10 ²	19.59	4.86
Apollo 12	3.0 x 10 ⁴	4.1 x 10 ³	1.1 x 10 ³	1.9 x 10 ²	3.69	0.20
Apollo 13	1.3 x 10 ⁴	2.1 x 10 ³	1.0 x 10 ³	2.0 x 10 ²	7.92	1.02
Apollo 14	4.8 x 10 ⁴	8.6 x 10 ³	1.7 x 10 ³	3.8 x 10 ²	3.63	1.41
SIA						
Apollo 10	2.2 x 10 ²	2.4 x 10 ¹	1.2 x 10 ¹	1.6 x 10 ¹	5.58	1.86
Apollo 11	8.3 x 10 ¹	2.4 x 10 ¹	6.5 x 10 ¹	2.4 x 10 ¹	78.31	4.82
Apollo 12	2.8 x 10 ¹	1.6 x 10 ¹	1.6 x 10 ¹	3.2 x 10 ¹	57.10	0.00
Apollo 13	1.6 x 10 ¹	8.0 x 10 ⁰	4.0 x 10 ⁰	0.0	25.00	0.00
Apollo 14	1.8 x 10 ²	5.6 x 10 ¹	2.0 x 10 ¹	0.0	10.90	0.00

¹ Average of three final sampling periods. Total area sampled was 180 sq. in.

² Percentage of total aerobic mesophilic microorganisms.

³ Total area sampled was 160 sq. in.

TABLE 9. COMPARISON OF THE LEVELS OF MICROBIAL CONTAMINATION DETECTED ON THE LUNAR MODULES OF APOLLO 10, 11, 12, 13, AND 14 SPACECRAFT.

Source	No. Microorganisms per square foot ¹				Percent ²	
	Aerobes	Anaerobes	Aerobic Spores	Anaerobic Spores	Aerobic Spores	Molds
Ascent Stage (interior)						
Apollo 10	1.8×10^5	1.0×10^5	3.7×10^2	3.2×10^1	0.21	0.002
Apollo 11	8.2×10^4	3.1×10^4	3.3×10^2	6.4×10^1	0.41	0.03
Apollo 12	4.9×10^4	1.3×10^4	7.2×10^1	2.4×10^1	0.15	0.16
Apollo 13	3.7×10^4	9.0×10^3	7.6×10^1	3.6×10^1	0.20	0.02
Apollo 14	1.1×10^5	5.5×10^4	3.1×10^2	6.0×10^1	0.29	0.02
Ascent Stage (exterior)						
Apollo 10	5.0×10^3	1.1×10^3	1.5×10^2	2.0×10^1	3.10	0.32
Apollo 11	5.1×10^3	1.2×10^3	1.8×10^2	3.6×10^1	3.50	2.68
Apollo 12	2.0×10^3	7.2×10^2	5.6×10^1	2.4×10^1	2.75	0.39
Apollo 13	2.7×10^3	6.1×10^2	3.6×10^1	1.2×10^1	1.33	0.74
Apollo 14	2.1×10^4	3.3×10^3	1.8×10^2	4.0×10^1	0.88	0.34
Descent Stage (exterior)						
Apollo 10 ³	1.6×10^4	1.1×10^4	5.1×10^2	5.4×10^1	3.13	1.08
Apollo 11	4.6×10^3	1.1×10^3	2.6×10^2	2.4×10^1	5.69	1.14
Apollo 12	1.1×10^4	5.2×10^3	1.4×10^2	2.8×10^1	1.25	0.44
Apollo 13	3.4×10^4	2.5×10^4	6.8×10^1	1.2×10^1	0.20	0.08
Apollo 14	1.1×10^5	6.0×10^4	2.3×10^2	4.0×10^1	0.21	0.04

¹ Average of three final sampling periods. Total area sampled was 180 sq. in.

² Percentage of total aerobic mesophilic microorganisms.

³ Total surface area sampled was 140 sq. in.

TABLE 10. TYPES OF MOLDS DETECTED ON THE APOLLO 14 SPACECRAFT.

Types	Number Isolated	Percent
Alternaria	3	8.8
Bipolaris	10	29.4
Chaetomium	2	5.9
Curvularia	6	17.7
Drechslera	4	11.8
Fusarium	1	2.9
Nigrospora	1	2.9
Penicillium	6	17.7
Pithomyces	1	2.9
Total 34		100.0

TABLE 11. COMPARISON OF TWO TYPES OF MEDIA FOR DETECTING FUNGI ON THE APOLLO
SPACECRAFT SURFACES.

Source	Apollo 10		Apollo 11		Apollo 12	
	TSA ¹	Mycophil ¹ Agar	TSA ¹	Mycophil ¹ Agar	TSA ¹	Mycophil ¹ Agar
Command Module ²	0.03	N.D.	0.13	N.D.	N.D.	N.D.
Instrument Unit ²	4.08	5.28	4.10	2.23	1.17	0.84
S-4B ²	2.86	2.23	3.25	2.81	0.42	2.50
SLA ² (Spacecraft Lunar Module Adapter)	0.03	N.D.	0.03	N.D.	N.D.	N.D.
<u>Lunar Module</u>						
Ascent Stage ² (interior)	0.06	N.D.	0.39	N.D.	0.56	0.84
Ascent Stage (exterior)	0.11	N.D.	0.96	1.11	0.06	N.D.
Descent Stage (exterior)	1.22	0.38	0.36	0.28	0.33	N.D.

¹ Average mold counts per in.² - Mean of three sampling periods.

² Samples taken from the interior surfaces of the spacecraft.

N.D. = None detected.

TABLE 12. COMPARISON OF THE RECOVERY OF AIRBORNE FUNGI IN THE MANNED SPACECRAFT OPERATIONS BUILDING (MSOB) BY DIFFERENT AIR SAMPLING METHODS:

Date	<u>Membrane Filters</u>	<u>Reyniers</u>
	Colonies/90 ft. ³ air	Colonies/180 ft. ³ air
7-7-70	3	6
7-8	8	5
7-13	3	4
7-14	15	16
7-15	27	47
7-20	3	2
7-21	4	4
7-22	5	7
7-27	4	10
7-28	4	4
7-29	0	2
8-3	2	7
8-4	1	9
8-5	6	7
Mean	.07 colonies per ft. ³	.05 colonies per ft. ³

TABLE 13. COMPARISON OF THE RECOVERY OF AIRBORNE FUNGI BY DIFFERENT AIR SAMPLING METHODS IN LABORATORY AREA.

Date	<u>Membrane Filters</u>	<u>Reyniers</u>
	Colonies/90 ft. ³ air	Colonies/180 ft. ³ air
5-18-70	0	19
5-19	0	9
5-20	0	19
5-25	8	24
5-26	19	23
5-27	0	20
6-2	39	45
6-3	31	63
6-8	27	34
6-9	20	29
6-10	10	13
6-15	13	20
6-16	16	15
6-17	7	14
6-22	9	11
6-23	14	19
6-24	16	10
6-29	17	22
6-30	7	16
7-1	13	38
Mean	.15 colonies per ft. ³	.13 colonies per ft. ³

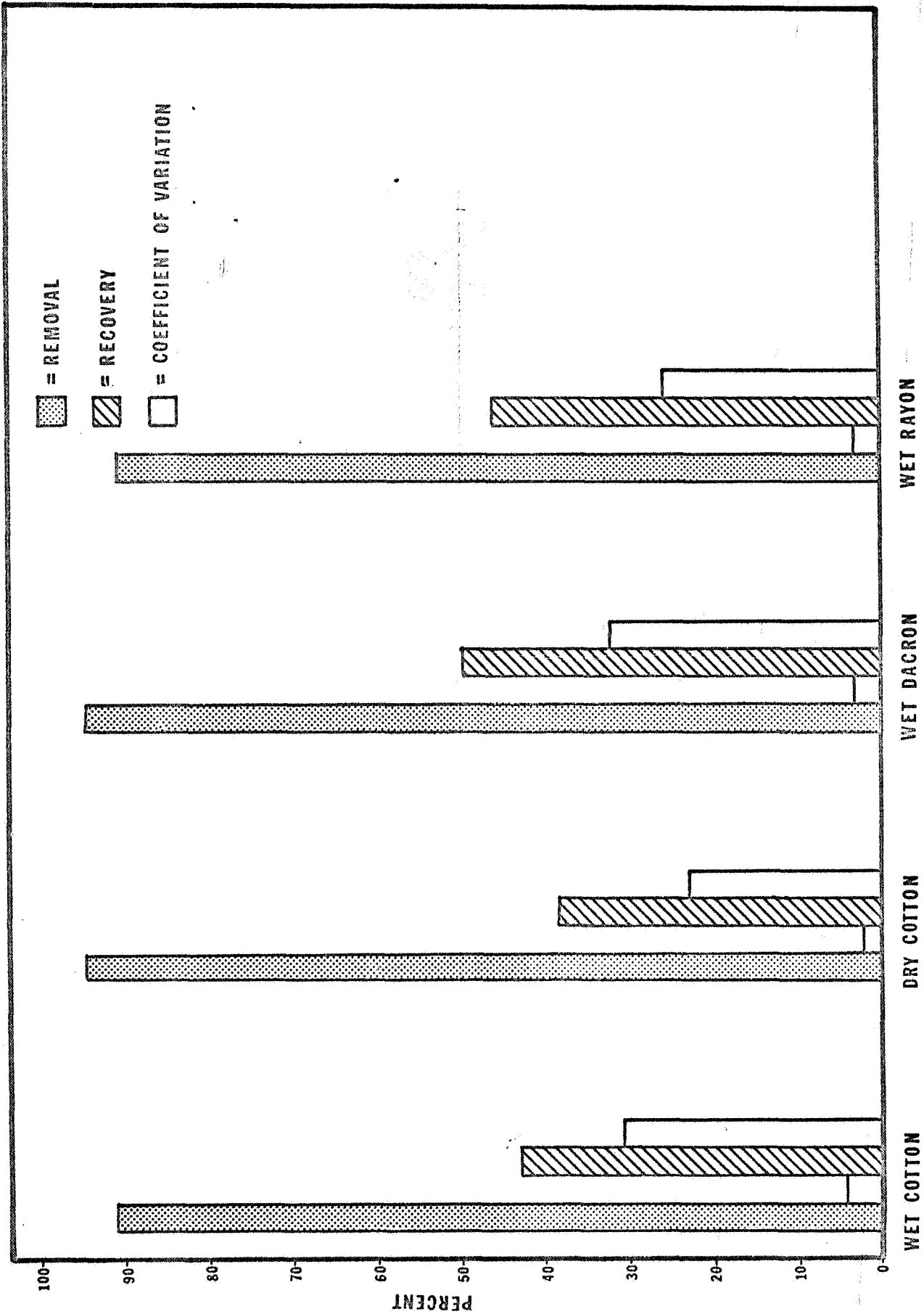


FIGURE 1. COMPARISON OF REMOVAL AND RECOVERY USING FOUR TYPES OF SWABS.

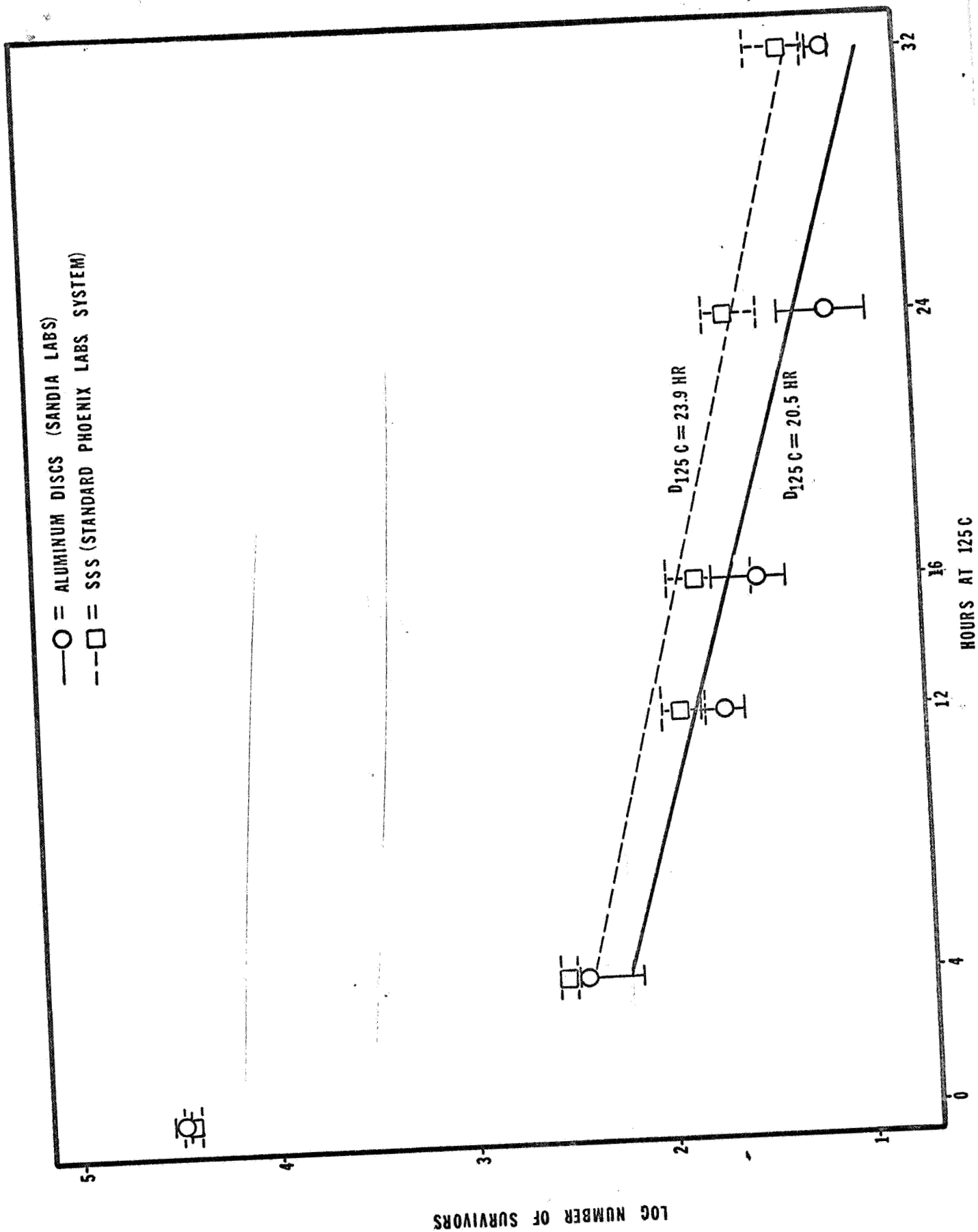


FIGURE 2. SURVIVAL OF KSC SOIL ON SANDIA ALUMINUM DISCS AND 1/2 in² STAINLESS STEEL STRIPS.

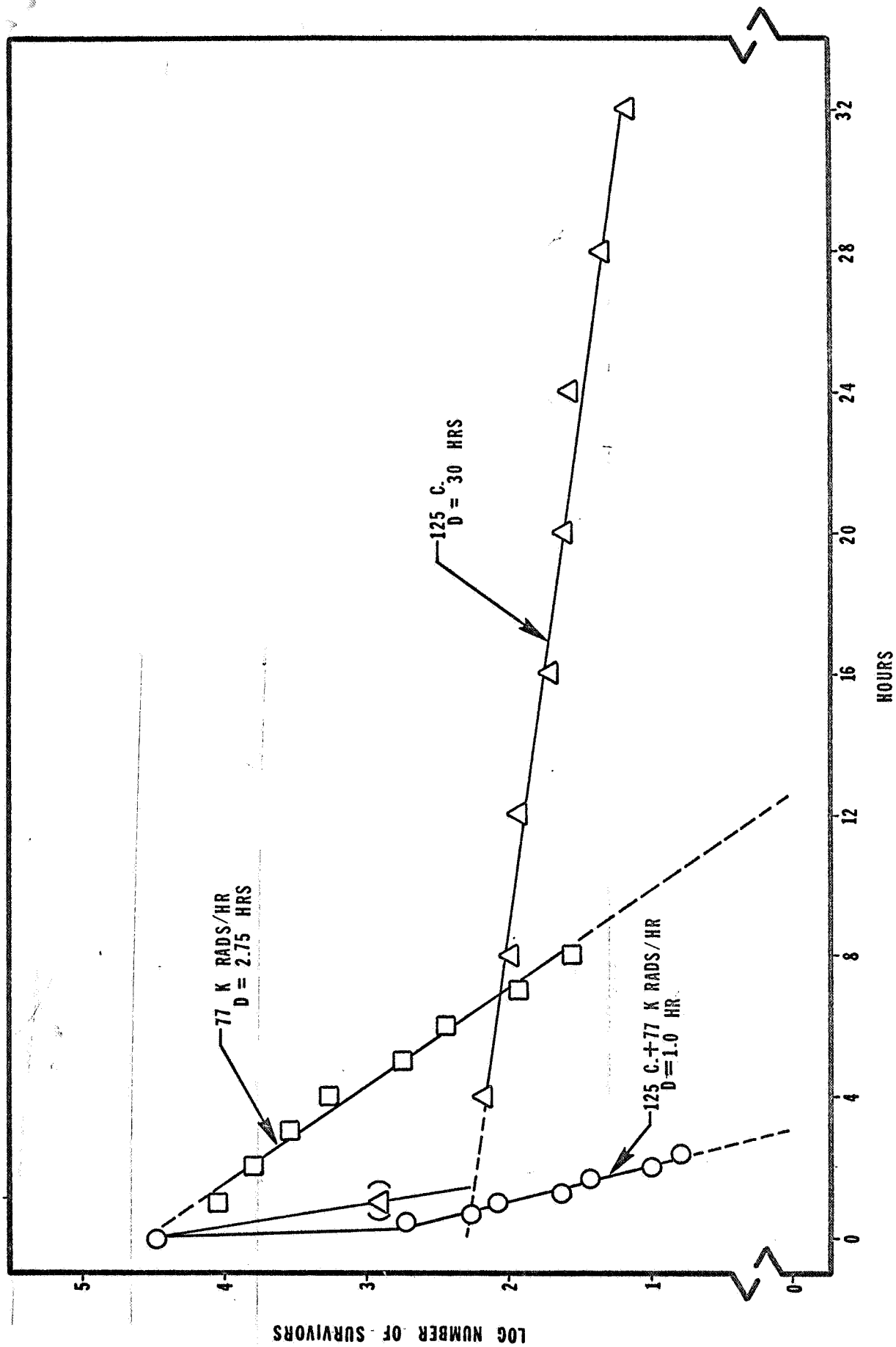


FIGURE 3. SURVIVAL OF KSC SOIL EXPOSED TO 125 C ALONE, GAMMA IRRADIATION ALONE, AND THERMORADIATION.